

1288

Price, As. 8.]

[Vol. 16A, Part VII, pp. 85 to 90.]

JOURNAL

OF THE

Indian Institute of Science

CONTENTS



I. STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS, PART V.
THE ALCOHOL SOLUBLE PROTEIN OF FENUGREEK
(*TRIGONELLA FOENUM GRAECUM*)

BY

Y. V. Sreenivasa Rao, B. N. Sastri and N. Narayana.

II. THE MUCILAGE OF FENUGREEK (*TRIGONELLA FOENUM
GRAECUM*)

BY

C. R. Harihara Iyer and B. N. Sastri.

28

Pa-11

JOURNAL
OF THE
INDIAN INSTITUTE OF SCIENCE

Volume 1 included 21 issues during 1914-18. Thereafter one volume was published annually until 1925, when Volumes 8 (A) and (B), followed by 9 (A) and (B) (1926) and 10 (A) and (B) (1927) appeared.

VOLUME 11 (A)—1928.

Parts I—XIX.

VOLUME 12 (A)—1929.

Parts I—XX.

VOLUME 13 (A)—1930.

Parts I—XV.

VOLUME 14 (A)—1931.

Part I.—Studies on the chemical composition and physical properties of plant tissue fluids.
Part I. Effects of age and environment on the tissue fluids of French beans (*Phaseolus Vulgaris*). By C. Narasimha Acharya and B. N. Sastri. Price, As. 12.

Part II.—The Photochemical Oxidation of Organic Hydrocarbons. Part II. Toluene, and the Oxidation of Benzaldehyde. By C. J. Kothari and H. E. Watson. Price, Rs. 1-4.

Part III.—I. Cantharidin from *Mylabris Pustulata* Fb., India. By B. H. Iyer and P. C. Guha.
II. The Fatty acids from oil of Cantharis (*Mylabris Pustulata* Fb., India) By B. H. Iyer and P. Ramaswami Ayyar. Price, Re. 1.

Part IV.—I. Amylase from Rice. II. Dialysis of Some Cereal Amylases. By Vinayak Narayan Patwardhan and Dattatreya Vishnu Karmarkar. Price, As. 12.

Part V.—Contributions to the Study of Spike-Disease of Sandal (*Santalum Album*. Linn.), Part XII. Ecology of Sandal. By M. Sreenivasaya and S. Rangaswami. Price, As. 8.

Part VI.—Studies in Electro-Culture. Part I. Introduction. By K. Venkata Giri, T. J. Mirchandani and V. Subrahmanyam. Studies in Electro-Culture. Part II. Influence of Electrical Treatment on the Germination of Barley and the Diastatic Activity of Malt. By K. Venkata Giri and V. Subrahmanyam. Price, Rs. 2.

Part VII.—The Origin and Nature of the Peaty Soils of Travancore. By T. R. Narayana Pillai and V. Subrahmanyam. Price, Re. 1.

Part VIII.—The Relation between Seeds and Micro-Organisms. By T. R. Sathe and V. Subrahmanyam. Price, Rs. 1-4.

Part IX.—The Reactivity of Dimethyldihydroresorcin. Part I. Condensation with Aromatic Aldehydes. By Gopal Chandra Chakravarti, Haripada Chattopadhyaya and Prafulla Chandra Ghosh. Price, Re. 1.

Part X.—The Reactivity of Dimethyldihydroresorcin. Part II. Behaviour towards Ortho-Nitro- and Ortho-Aminobenzaldehydes. By B. H. Iyer and G. C. Chakravarti. Price, Re. 1.

JOURNAL

OF THE

Indian Institute of Science

CONTENTS

- I. STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS, PART V.
THE ALCOHOL SOLUBLE PROTEIN OF FENUGREEK
(*TRIGONELLA FOENUM GRAECUM*)

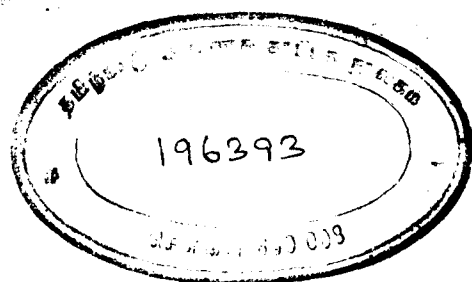
BY

Y. V. Sreenivasa Rao, B. N. Sastri and N. Narayana.

- II. THE MUCILAGE OF FENUGREEK (*TRIGONELLA FOENUM
GRAECUM*)

BY

C. R. Harihara Iyer and B. N. Sastri.



52
A, d, m 2, N1413
N33.16A.7

**STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.
PART V. The Alcohol Soluble Protein of Fenugreek
(*Trigonella Fœnum Græcum*).**

By Y. V. Sreenivasa Rao, B. N. Sastri and N. Narayana.

In a previous communication Sreenivasa Rao and Sreenivasaya (*Jour. Indian Inst. Science*, 1932, **15A**, 122) have reported their findings on the albumin and the globulin of fenugreek. The present paper deals with the prolamin from the same source.*

EXPERIMENTAL.

The flour was first extracted with ether in a Soxhlet and the residue (3 Kg.) stirred up with 70 per cent. alcohol (9 L) in the cold for six hours. The mixture was then warmed on the water bath and maintained under reflux for 1 hour at the boiling temperature. The hot suspension was then filtered and the extract on cooling in the ice chamber deposits a white solid. After filtration, the extract was concentrated at 40° under reduced pressure. The concentrate (2 L) was allowed to stand overnight at room temperature when a white solid separated. The precipitate was washed consecutively with distilled water, 90 per cent. alcohol and ether. It was then dried, powdered to pass through a 100-mesh sieve and preserved in a desiccator over calcium chloride (Preparation I; yield 5 g.)

On treating the concentrated alcoholic extract (1.5 L) with acetone (8 L), a copious precipitate was obtained a large portion of which was found to be soluble in water. The insoluble portion was separated on the centrifuge, washed repeatedly with distilled water and dehydrated with alcohol and ether. On drying, a light, white solid was obtained (Preparation II; yield, 4 g.)

Both the above-mentioned preparations gave all the reactions for proteins and contained tyrosine, tryptophan, sulphur and phosphorus. On analysis they gave the following results. (Table I.)

* Since writing this paper a preliminary note on the prolamin of fenugreek prepared by a different method by A. Hassan and M. K. A. Basha has appeared in the *Biochemical Journal* (1932, **26**, 1843.) That publication does not, however, relate to the composition of the prolamin.

TABLE I

Preparation	PERCENTAGES				
	Moisture	Ash	P ₂ O ₅ ¹	Ash and moisture free	
				Nitrogen	Sulphur ²
I	3.0	0.99	0.50	14.03	1.12
II	9.6	1.10	0.41	14.08	0.99

¹ Micro method of Pregl.² Micro-combustion method of Carius.

The two preparations were then analysed by the method of Van Slyke as modified by Plimmer and his co-workers (*Biochem. J.*, 1925, **19**, 1004; 1927, **21**, 247). Tyrosine, tryptophan and cystine were estimated separately on the whole protein according to Folin and Marenzi (*J. Biol. Chem.*, 1929, **83**, 89).

The distribution of nitrogen is given in the following table.—

TABLE II

Form of Nitrogen	Percentages of total nitrogen			
	Preparation			
	I		II	
Acid insoluble Melanin	...	0.9	...	1.1
Acid soluble Melanin	...	0.4	...	0.5
Amide	...	20.2	...	20.0
Basic	...	5.8	...	5.6
Arginine	...	3.1	...	2.9
Histidine	...	0.7	...	0.7
Cystine	...	1.4	...	1.3
Lysine	...	0.7	...	0.7
Non-basic:	...	72.7	...	72.8
Amino	...	70.2	...	70.4
Non-amino	...	2.5	...	2.4

A noteworthy feature of the prolamin from fenugreek is its low basic nitrogen content in which respect it is comparable only with eleusine from ragi (Hoffman and Gortner, Coll. Symp. Monograph, 1925, Vol. 2, 246; Niyogi, Narayana and Desai, private communication). 196393

In Table III are given the percentages of the more important amino acids as estimated for the whole protein. The corresponding figures for ragi prolamin are cited for comparison.

TABLE III

		Source of prolamin		Method
		Fenugreek	Ragi	
Arginine	...	2.3	2.5	Direct
Histidine	...	0.4	1.4	Van Slyke
Lysine	...	0.5	0.5	„
Cystine	...	3.0	2.5	Folin and Marenzi
Tyrosine	...	4.3	5.3	„
Tryptophan	...	2.4	1.6	„

SUMMARY.

The two preparations of the prolamin from fenugreek obtained by different methods were found to have very nearly the same composition. The prolamin is characterised by a low basic nitrogen content and high percentages of cystine and tryptophan and thus resembles the alcohol soluble protein of ragi isolated by one of us.

In conclusion, we wish to express our thanks to Professor V. Subrahmanyam for his kind interest in the progress of the work.

Department of Biochemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 28-4-33.]

3L
A, d, m2, N14 IS
N 33 16 A.7

THE MUCILAGE OF FENUGREEK (*TRIGONELLA FÆNUM GRAECUM*).

By C. R. Harihara Iyer and B. N. Sastri.

In a recent communication on the subject (*Biochem. J.*, 1932, **26**, 255) Daoud has described the mucilage of fenugreek as the silico-phosphoric ester of manno-galactan. His method of preparation consists in the addition of alcohol to an aqueous extract of fenugreek bran till the final concentration of alcohol reaches 30 per cent. The precipitate thus obtained is redissolved in water and reprecipitated by addition of alcohol.

The product so obtained does not however appear to have been subjected to any further purification. The quantitative relationships between phosphorus and silicon or between either of those elements and total carbohydrates have not been examined. The present authors and their associates have been engaged on the chemical examination of fenugreek for the past few years and the object of this communication is to place on record a few important observations on the purification and chemical composition of the mucilage.

Preparation :—The mucilage was first precipitated from the aqueous extract of bran in a manner similar to that described by Daoud (*loc. cit.*). The preparation thus obtained was dissolved in water and reprecipitated by alcohol, the process being repeated several times to obtain a pure product. It was observed that the ash content of the preparation decreased steadily on purification as may be seen from the analyses of successive precipitates (Table I).

TABLE I

Precipitate as obtained in order	Percentages		
	Ash	Phosphorus *	Nitrogen
1	1.57	0.24	...
2	0.68	0.07	0.53
3	trace	0.02	0.11

* According to the micro-method of Pregl.

The results indicate that one or more compounds containing nitrogen and phosphorus occur as impurities in association with the mucilage.

Electro-dialysis.—In order to eliminate crystalloidal impurities mechanically carried down by the mucilage, an aqueous extract of the mucilage (2nd precipitation) was subjected to electro-dialysis using the main laboratory supply (110 D.C.) as the source of current. Three thick arc carbons served as anode and a platinum strip (1½ sq. in.) was used as cathode. The liquid in the middle cell was periodically stirred, and the dialysis continued for a period of 4 days, when the conductivity reached almost that of distilled water. The product obtained on precipitating the dialysed extract contained the same percentages of phosphorus and nitrogen as the undialysed one, thereby showing that the impurities were not crystalloids.

Saturation with Magnesium sulphate.—In view of the previous observations of Sreenivasa Rao and Sreenivasaya that phosphorus is associated with the water-soluble protein of fenugreek (*J. Indian Inst. Sci.*, 1932, **15A**, 122), further purification of the mucilage was directed towards obtaining a protein-free product. An aqueous solution of the mucilage was saturated with magnesium sulphate and the precipitate thus obtained separated by centrifuging. The mucilage was precipitated from the centrifugate by addition of alcohol. The precipitate was washed successively with 50 per cent. alcohol, absolute alcohol and finally, ether. The white product thus obtained possessed all the properties of the original mucilage but was entirely free from both phosphorus and nitrogen.

The above observations show clearly that the mucilage itself does not contain any phosphorus or nitrogen but occurs associated with one or more impurities which are either proteins or some other types of compounds that can be removed by protein precipitants.

Removal of impurity by treatment with boiling alcohol.—An aqueous solution of the crude mucilage was treated with alcohol to a concentration of 30 per cent. The suspension thus obtained was refluxed over a boiling water-bath for 8 hours and allowed to stand at the room temperature for 48 hours. The precipitate was then separated, extracted with water and the mucilage precipitated from the aqueous extract with alcohol. Analysis of the precipitate showed that its phosphorus content was almost negligible (0.001 per cent.) thus lending further support to the suggestion that the impurity resembled proteins in behaviour and was denatured and coagulated by boiling alcohol.

Hydrolysis of the mucilage.—On hydrolysis with acids, the mucilage yielded mannose and galactose in equal proportions as has been reported by previous workers. Elementary analysis of the purified product gave the following percentages.—C, 44.3; H, 6.2; and O, 49.5. The results would thus show that the mucilage is a mannogalactan with the empirical formula, $C_6H_{10}O_8$.

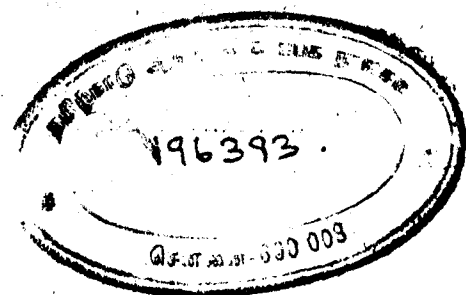
SUMMARY.

The mucilage from fenugreek is usually associated with a nitrogenous impurity which can be removed either by repeated dissolution in water and reprecipitation with alcohol or by saturation with magnesium sulphate. The purified mucilage is a mannogalactan.

The authors' thanks are due to Mr. M. Sreenivasaya for many helpful suggestions.

Department of Biochemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 28-4-33.]



72

A, d, m, N14 IS

N33 16A.7

VOLUME 15 (A)—1932.

- Part I.—Salt Stains on South Indian Hides and Skins. By V. N. Patwardhan and M. Subramania Sastry. Price, As. 12.
- Part II.—Chemical Examination of the Root-Bark of *Plumbago Rosea*, Linn. By M. C. Tummin Katti and V. N. Patwardhan. Price, As. 8.
- Part III.—Dilatometric Studies in Enzyme Action. Part II. Contraction Constants of Enzyme-Substrate Reactions. By M. Sreenivasaya and H. B. Sreerangachar. Price, As. 12.
- Part IV.—Aryl Di- and Poly-Stibinic Acids, Distibinous Oxides and Distibino-Compounds. By Sohrab M. Mistry and Praphulla Chandra Guha. Price, Re. 1.
- Part V.—Bhadravati Wood-Tar and its Utilization. By Y. K. Raghunatha Rao, B. Sanjiva Rao and H. E. Watson. Price, Re. 1.
- Part VI.—The Equilibrium between Dimethyl Ether, Methyl Alcohol and Water. By N. G. Gajendragad, S. K. Kulkarni Jatkar and H. E. Watson. Price, Re. 1.
- Part VII.—Studies in Indian Essential Oils. I. Essential Oil from Leaves of *Litsæ Zeylanica* Linn. By B. Sanjiva Rao. II. Essential Oil from Flower-Heads and Stalks of *Andropogon Kuntzeanus*, Hack, *Var. Foveolata*, Hack. By B. Sanjiva Rao. III. Essential Oil from Leaves of *Thymus Serpyllum*, Linn. By Jagjit Singh and B. Sanjiva Rao. IV. Essential Oil from Leaves of *Cinnamomum Zeylanicum*, Breyn. By (the late) V. P. Shintre and B. Sanjiva Rao. Price, Re. 1.
- Part VIII.—Organic Manure from Sewage, Town Refuse and Waste Vegetation. By J. Jagannatha Rao and V. Subrahmanyam. Price, Rs. 1-8.
- Part IX.—I. Studies in Enzyme Action. Part V. Ageing of Amylases in Aqueous Solution. By K. Venkata Giri and V. Subrahmanyam. II. Studies in Enzyme Action. Part VI. Heat Inactivation of Pancreatic Amylase. By K. Venkata Giri. III. Studies in the Proteins of Indian Foodstuffs. Part IV. The Proteins of Fenugreek (*Trigonella Foeniculum Graecum*). By Y. V. Srinivasa Rau and M. Sreenivasaya. Price Rs. 1-8.
- Part X.—Studies in Bridge Formation. Part I. Attempts to Synthesise Bicyclic Terpene Derivatives by a New Method. By P. P. Patel and P. C. Guha. Part II. Formation and Stability of a Bridged cycloHexenone, 1-Dimethylethane-1:2:3:4 III 1:2:3:4 cyclobutane-2-one. By P. S. Mayuranathan and P. C. Guha. Price, Re. 1.

VOLUME 15 (B)—1932.

- Part I.—A New Permeameter. By K. V. Karantha. Price, Rs. 1-8.
- Part II.—Measurement of Power Factor and Loss in Dielectrics. By T. J. Mirchandani, G. Yoganandam, S. K. Roy and N. V. Narayanaswami. Price, Rs. 1-4.
- Part III.—Transients in Negative Constant Series Circuits. By L. C. Verman. Price, Rs. 1-4.
- Part IV.—Telephony by Carrier and One Side Band. By S. P. Chakravarti. Price, Re. 1.

VOLUME 16 (A)—1933.

- Part I.—Rhaponticin, and Anthraquinone Derivatives from *Rheum Emodi*, Wall. (Indian, or Himalayan Rhubarb). By M. Ghouse Mohiuddin and M. C. Tummin Katti. Price, As. 12.
- Part II.—Constitution of the So-Called Dithiourazole of Martin Freund. Part V. Isomerism of Hydrazodithiodicarbonamides, Iminothiolthiobiazoles and Di-R-Iminothiobiazoles. By Shaha L. Janniah and P. C. Guha. Part VI. Isomerism of Hydrazomonothiodicarbonamides, Iminothiobiazolones and Monothiourazoles. By S. L. Janniah and P. C. Guha. Price, Re. 1-4.

Part III.—Indian Medicinals Plants. Part I. *Withania Somnifera*. Part II. *Swertia Chirreta*. By D. N. Majumdar and P. C. Guha. Price, As. 12.

Part IV.—I. The Colouring Constituents of Alkanet Root (*Anchusa Tinctoria*, Lam.). Part I. The Constitution of Alkannin. By Mangesh V. Betrabet and Gopâl Chandra Chakravarti. II. Note on the Constituents of the Wax from Alkanet Root. By M. V. Betrabet and G. C. Chakravarti. Price, Re. 1.

Part V.—The Velocity of Alcoholysis of Esters of Dibasic Acids. By Z. H. Patel and H. E. Watson. Price, As. 12.

Part VI.—I. Dilatometric Studies in Enzyme Action. Part III. Contraction Constants of Enzyme-Substrate Reactions. By H. B. Sreerangachar and M. Sreenivasaya. II. Physiological Products of the Lac Insect. Part I. A Preliminary Investigation. By N. K. Ranga Rao and M. Sreenivasaya.

Part VII.—I. Studies in the Proteins of Indian Foodstuffs, Part V. The Alcohol Soluble Protein of Fenugreek (*Trigonella Foenum Graecum*). By Y. V. Sreenivasa Rao, B. N. Sastri and N. Narayana. II. The Mucilage of Fenugreek (*Trigonella Foenum Graecum*). By C. R. Harihara Iyer and B. N. Sastri.

VOLUME 16 (B)—1933.

Part I.—Taylor's Frequency Tripler. By P. Rajagopal Pillai and F. N. Mowdawalla. Price, Re. 1.